

ABNORMAL MENSTRUAL BLEEDING: A ROLE FOR THE PROSTAGLANDINS

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"And if a woman have an issue, and her issue
in her flesh be blood, she shall be put apart
seven days: and whosoever toucheth her shall
be unclean until the even".

Leviticus, Ch15, V 19.

Since ancient times menstruation has been very much a taboo subject, poorly understood and enshrouded in mystery. However, over the years much interest has been aroused by this monthly endometrial shedding, partly out of a desire to discover something of its underlying mechanisms, but also as a result of the vast clinical problem that disorders of menstruation can present, for it has been estimated that excessive menstrual blood loss may affect up to 20% of women during their reproductive years (Jacobs et al, 1965; Hallberg et al, 1966).

Heavy menstrual bleeding, or menorrhagia, may be the result of organic diseases such as endometrial polyps or fibroids, but in the majority of instances, no such underlying lesion can be found, in which case the diagnosis of dysfunctional uterine bleeding can be made (Novak et al, 1965). In some circumstances, and especially at the extremes of the reproductive career, this dysfunctional bleeding may be the result of a disturbance of ovarian function (Fraser et al, 1973; Van Look et al, 1977), however, in most women with regular but heavy periods, no impairment of the hypothalamo - pituitary - ovarian axis can be demonstrated (Haynes et al, 1979). In consequence, much recent work has focussed on the endometrium itself, for it is possible that local factors may play an important part in the mechanisms controlling menstruation and its disorders.

Observing the growth and degeneration of endometrial explants in the anterior chamber of the eye of the monkey, Markee (1940) suggested that the

initial process occurring at the onset of menstruation was one of intense vasoconstriction of the spiral arterioles, with accompanying vasodilatation of the surrounding vessels, and with the discovery that the endometrium contains large quantities of the prostaglandins E_2 and $F_2\alpha$ (Pickles et al, 1965), the hypothesis that these vasoactive compounds may contribute to the control of menstrual blood loss could be proposed.

It is now evident that excessive menstrual blood loss is associated with changes in the prostaglandin (PG) production of the uterus (Smith et al, 1981 a & b; Willman et al, 1976). Endometrium from women with ovular dysfunctional uterine bleeding synthesises more PGE_2 than that of normal women, and markedly enhances the myometrial production of PGI_2 . (PGI_2 or prostacyclin, a vasodilatory substance, and the most potent inhibitor of platelet aggregation yet discovered (Moncada & Vane, 1980), is converted to 6oxo $PGF_1\alpha$, which is the major product synthesised when arachidonic acid is incubated with rat or sheep uteri (Jones et al, 1977)). In women with anovular dysfunctional bleeding, excessive menstrual loss is associated with a relative deficiency of $PGF_2\alpha$ synthesis (Smith et al, 1982). There is in fact an inverse relationship between the ratio of $PGF_2\alpha$: PGE_2 and the amount of blood lost, and it may be, therefore, that menstrual loss is determined by the relative synthesis of prostaglandins with mainly vasoconstrictor properties on the one hand ($PGF_2\alpha$) as opposed to those with vasodilatory properties (PGE_2 , PGI_2) on the other (Baird et al, 1981).

This manuscript presents further evidence suggesting a role for the prostaglandins in the pathogenesis of menorrhagia.

METHODS

Patient Recruitment

Fifty parous women were recruited for study from the Gynaecological Out Patient Department of the Royal Infirmary, Edinburgh. All suffered from menorrhagia, diagnosed subjectively, for which no organic cause had been found. Recruits were instructed to collect their soiled sanitary protection for two cycles, in order to assess their menstrual loss objectively, and they were also asked to collect early morning urine samples three times weekly, for total oestrogen and pregnanediol estimation. In addition, an endometrial biopsy was performed on 13 women in the mid-luteal phase of the second cycle, in order to measure endometrial prostaglandin concentrations. These biopsies were performed without anaesthesia, using a Sharmann Curette.

Written informed consent was obtained from all women, and approval for the study was granted by the Ethical Sub-Committee in Reproductive Medicine.

Measurement of Menstrual Blood Loss

Menstrual blood loss was assessed objectively using a modification of the alkaline haematin method of Hallberg & Nilsson (1964). Soiled pads and tampons were placed in molar NaOH and thoroughly mixed. 24 hours later, an aliquot was taken, and after filtration, its optical density was measured. Menstrual blood loss was then calculated by comparing this with the optical density of a peripheral blood sample, similarly processed. The diagnosis of menorrhagia was made if the menstrual blood loss exceeded 50 mls per cycle.

Tissue Collection and Prostaglandin Measurement

Endometrial biopsies were collected on ice into modified 199 medium (Flow Laboratories, U.K.). After transport to the laboratory within 5 minutes, the tissue was blotted dry on a gauze swab, weighed, and immediately placed in ice cold ethanol. The tissue was then homogenised using a polytron homogeniser, and centrifuged at 1500 g for 10 minutes at 4°C. The supernatant was aspirated, dried under nitrogen, and stored at -20°C, until assayed. Endogenous concentrations of endometrial prostaglandins were measured using standard radioimmunoassay techniques (see Abel et al, 1980). Antibodies raised against PGE₂ and PGF₂α (Dr J. Hennam, Kings College, London) and 6oxoPGF₁α (Dr J.A. Salmon, Wellcome Research Laboratories, Beckenham) were employed - and as the antibody against PGE₂ cross reacted significantly with PGE₁, the value for the concentration of PGE presented includes prostaglandin from both the 1 and 2 series. Inter and intra assay coefficients of variation, taken over 10 sequential assays and in duplicate 10 times within the same assay, were 12.2% and 14.4% for PGF₂α, and 10.1% and 11.2% for PGE. The intra assay coefficient of variation for 6oxoPGF₁α was 14.5%.

Combined Incubations of Endometrium and Myometrium.

A further 6 women suffering from benign uterine disease were admitted for abdominal hysterectomy in the luteal phase of the cycle. At operation, samples of both endometrium and myometrium were placed in ice-cold 199 medium and transported to the laboratory. After blotting and weighing both types of tissue, a myometrial homogenate was prepared in 199 medium, at a final concentration of 10mg/ml. Thereafter, small pieces of endometrium (approx. 5mg) were placed in either 1ml of 199 medium or 1ml of the myometrial homogenate, and these samples were incubated at 37°C in a water bath for 1 hour, with gentle shaking. 1ml samples of the myometrial homogenate alone, and control samples

of 199 medium were also processed in similar fashion. Following the incubation, the pieces of endometrium were removed, and the samples were stored at -20°C until assayed. Prostaglandin measurement by radioimmunoassay was performed as previously detailed.

Endometrial Dating

In all experiments, a portion of endometrium was placed in formol saline for histological dating (Noyes et al, 1950). The occurrence of ovulation was indicated by the presence of secretory endometrium, along with a pregnanediol/creatinine ratio greater than 1.0 mg/gm in the early morning urine specimens.

Statistical Analysis

Non parametric statistical tests were employed (Siegel, 1956). Significance between independent samples was assessed by the Wilcoxon rank sum test, and correlation analysis was performed using the Spearman rank correlation test.

RESULTS

Patient Characteristics and Menstrual Blood Loss

Of the 50 women recruited for menstrual blood loss assessment, 2 became menopausal during the study and a further 3 failed to collect their soiled sanitary protection adequately. The measured blood loss of the remaining 45 women is shown in Figure 1. It can be seen that there was a wide range of menstrual losses, and that in this selection of women, all with a subjective complaint of menorrhagia, almost 50% actually experienced a monthly blood loss of less than 50 mls.

The characteristics of the two groups of women (ie. those with and without objective menorrhagia) are presented in Table 1. There was no difference between the groups in terms of age or parity, nor were there differences in the duration of bleeding or cycle length. Although the mean haemoglobin concentration of the menorrhagic group was significantly lower than that of the other group, the value of 12.6 ± 0.2 g/dl was well within the range of normality. Furthermore, there was no compensatory increase in bone marrow turnover, as indicated by the reticulocyte count, to explain the normal haemoglobin concentration in the women with heavy blood loss.

As regards ovarian function, most of the women in both groups exhibited ovular menstrual cycles (Table 1).

Endometrial Prostaglandin Concentrations

The relationship between the endometrial prostaglandins and objectively assessed menstrual blood loss is indicated in Figure 2. This reveals that there was a significant correlation between the degree of menstrual blood loss and both PGE and $\text{PGF}_2\alpha$ ($p < 0.02$ and $p < 0.05$ respectively). Although there was an increase in endometrial $6\text{-oxoPGF}_1\alpha$ concentration in relation to menstrual loss, this relationship did not reach statistical significance. However, the "total" prostaglandin concentration ($\text{PGE} + \text{PGF}_2\alpha + 6\text{-oxoPGF}_1\alpha$) did correlate with the degree of blood loss ($p < 0.05$).

Combined Endometrium and Myometrium

The characteristics of the 6 patients are seen in Table 2. All were in the secretory phase of the cycle, and all showed luteal function both histologically and biochemically. Each of the women complained of heavy periods, with concomitant dysmenorrhoea in 2 cases, however, their menorrhagia was not assessed objectively.

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Endometrial prostaglandin production, both alone and in combination with myometrium, is presented in Figure 3. After correcting for the production of prostaglandin by the myometrial homogenate alone, the value for the combined incubate is expressed in pg/mg endometrium per hour. As can be seen, there was an increase in the production of $6\alpha\text{-PGF}_{1\alpha}$ by the endometrium when incubated in combination with myometrium, and, in addition, when the production was expressed as a percentage of "total" prostaglandin this increase in $6\alpha\text{-PGF}_{1\alpha}$ was associated with a significant decrease in the proportion of PGE formed.

DISCUSSION

The principle of objective assessment is fundamentally important in scientific research, and this principle is well illustrated when considering the problem of menorrhagia. In the present study 19 (42%) of 45 women with a subjective complaint of heavy menses had a measured blood loss of less than 50 mls, and similar results have been found elsewhere (I.S. Fraser and R. Markham, personal communication). Furthermore, there was no difference in the duration of bleeding or cycle length in women with heavy periods compared with those with a normal blood loss, and this again serves to highlight the pitfalls associated with defining menorrhagia on a purely subjective basis.

The figure of 50mls as the upper limit of normal is taken from a previous study assessing the blood loss of a group of patients without menstrual abnormality, who were presenting for laparoscopic sterilisation (Smith et al, 1983). Other workers have used 80mls as the "cut-off" point, based on the data of Hallberg et al. (1966), who found that this degree of blood loss represented the upper 10th centile of the menstrual blood loss range in a large population, and that this was the level at which iron deficiency problems began to emerge. Although it might be thought that defining the level of menorrhagia at 50 mls

rather than 80 mls could introduce a "masking effect", thereby accounting for the mean haemoglobin concentration in our menorrhagic group, this was not found to be the case, for the mean haemoglobin concentration in those women in the present study with a blood loss greater than 80 mls, was also within normal limits (12.3 ± 0.3 g/dl, $n = 13$).

16% of the low blood loss group and 12% of the menorrhagic women had anovular cycles. This would agree with the previously cited data that the pattern of ovarian steroid secretion is normal in the majority of women suffering from menorrhagia (Haynes et al, 1979).

A relationship between menstrual blood loss and the vasodilatory PGE, reflected by an increase in the $\text{PGE}:\text{PGF}_2\alpha$ ratio in menorrhagic women, has been suggested by a number of studies (Baird et al, 1981; Smith et al 1983), and the present results showing a correlation between this prostaglandin and the degree of menstrual loss would further support this idea. On the other hand, the finding that menstrual loss is also correlated with $\text{PGF}_2\alpha$ and "total" PG would appear to be at variance with the hypothesis of a balance mechanism between the vasoconstrictory and vasodilatory prostaglandins.

The precise relationship between the prostaglandins and menstrual loss is not yet clear - Rees, for example, has reported an increase in the ratio of $\text{PGF}_2\alpha:\text{PGE}$ in the menstrual fluid of women suffering from menorrhagia (Rees, 1983). Without doubt, great care must be taken when comparing the results of different studies, due to many variable factors such as patient recruitment, tissue collection and experimental methods, nevertheless, the suggestion that menstrual blood loss might be related to total endometrial prostaglandin concentration is in fact an attractive one. It would provide a logical explanation for the finding that menstrual loss is improved in some women by the indiscriminate inhibition of both the vasodilatory and vasoconstrictory prostaglandins, by the use of cyclo-

oxygenase inhibitors such as mefenamic acid (Anderson et al, 1976), and in addition, recent work has shown that there is an increased availability of arachidonic acid, and therefore, presumably prostaglandin, in the endometrium of women with menorrhagia (Kelly et al, 1984).

It has previously been demonstrated that the endometrium from women with menorrhagia has a greater capacity than normal endometrium to generate $6\alpha\text{-PGF}_{1\alpha}$, when incubated in combination with control myometrium (Smith et al, 1981b). This increased $6\alpha\text{-PGF}_{1\alpha}$ production was shown by incubating the tissues with exogenous precursor in the form of ^{14}C -labelled arachidonic acid. In our experiments, combinations of endometrium and myometrium from the same patients were tested, with the endometrium itself acting as the source of precursor. There was an increase in $6\alpha\text{-PGF}_{1\alpha}$ production when the tissues were incubated together, suggesting that the endometrium could provide precursor endoperoxide for prostaglandin production by the myometrium. As the major product of the arachidonic acid cascade in the myometrium is PGI_2 , the physiological result of delivering such precursor to the myometrium would be an increase in vasodilatation and a decrease in platelet aggregation, and both of these mechanisms might favour increased menstrual loss.

The variable degree by which the $6\alpha\text{-PGF}_{1\alpha}$ production increased in the present experiments might have been related to the level of menstrual loss, however, further studies recruiting women with objectively assessed periods would be necessary to test this hypothesis.

In conclusion, the possible inter-relationships between the prostaglandins and the uterine vasculature are summarised in Figure 4. The stimulus that initiates menstruation could lead to the liberation of free arachidonic acid,

presumably via phospholipase activation, and the arachidonic acid in turn could be converted into the various prostaglandins via the cyclo-oxygenase pathway. The resultant balance between the pharmacological actions of these prostaglandins might play a part in the control of the endometrial and myometrial vessels, and therefore the degree of menstrual blood loss. However, if excess free arachidonic acid were released, and thus increased precursor endoperoxide available, not only might the synthetic pathway towards $\text{PGF}_{2\alpha}$ (via PGF reductase) become saturated, leading to a diversion of endoperoxide towards PGE non-enzymatically, but also more PGI_2 could be formed, both in the endometrium and, in addition, in the large muscle mass of the myometrium. This increase in prostacyclin production could have important implications in the pathogenesis of menorrhagia.

The prostaglandins would appear to play a part, at least in some women, in the processes associated with menstrual bleeding, although other events, such as fibrinolysis, may also have a role. It is hoped that a better understanding of the mechanism underlying the menses might lead to a more rational approach to the clinical management of menstruation and its disorders.

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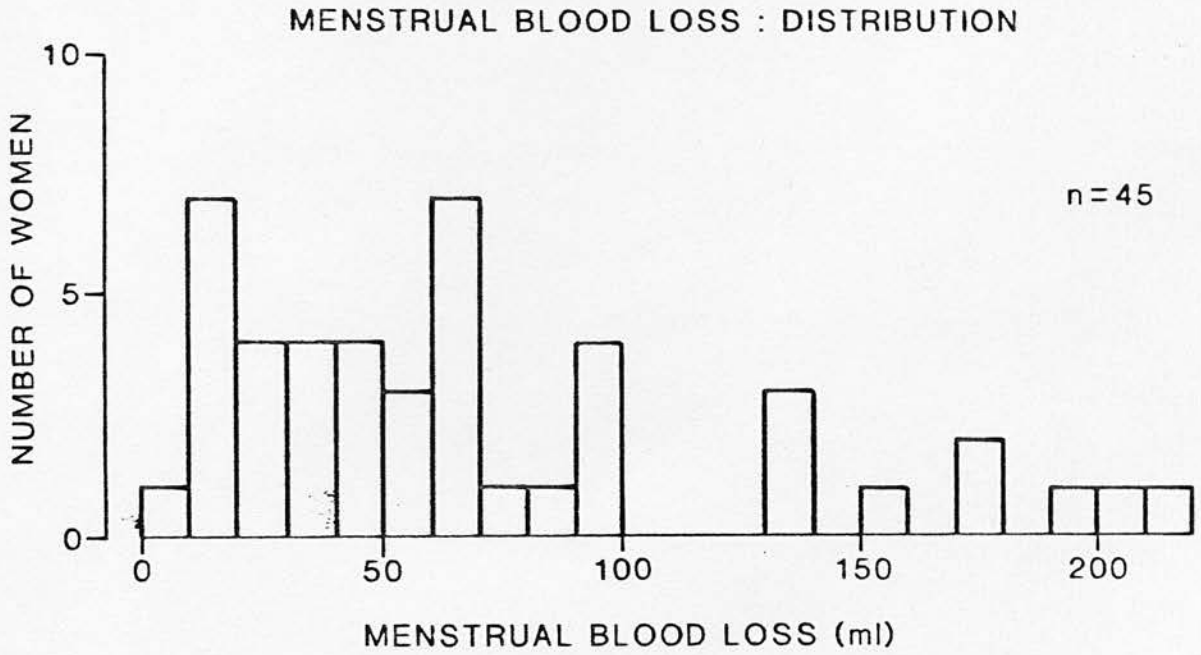


Figure 1. Menstrual blood loss distribution.

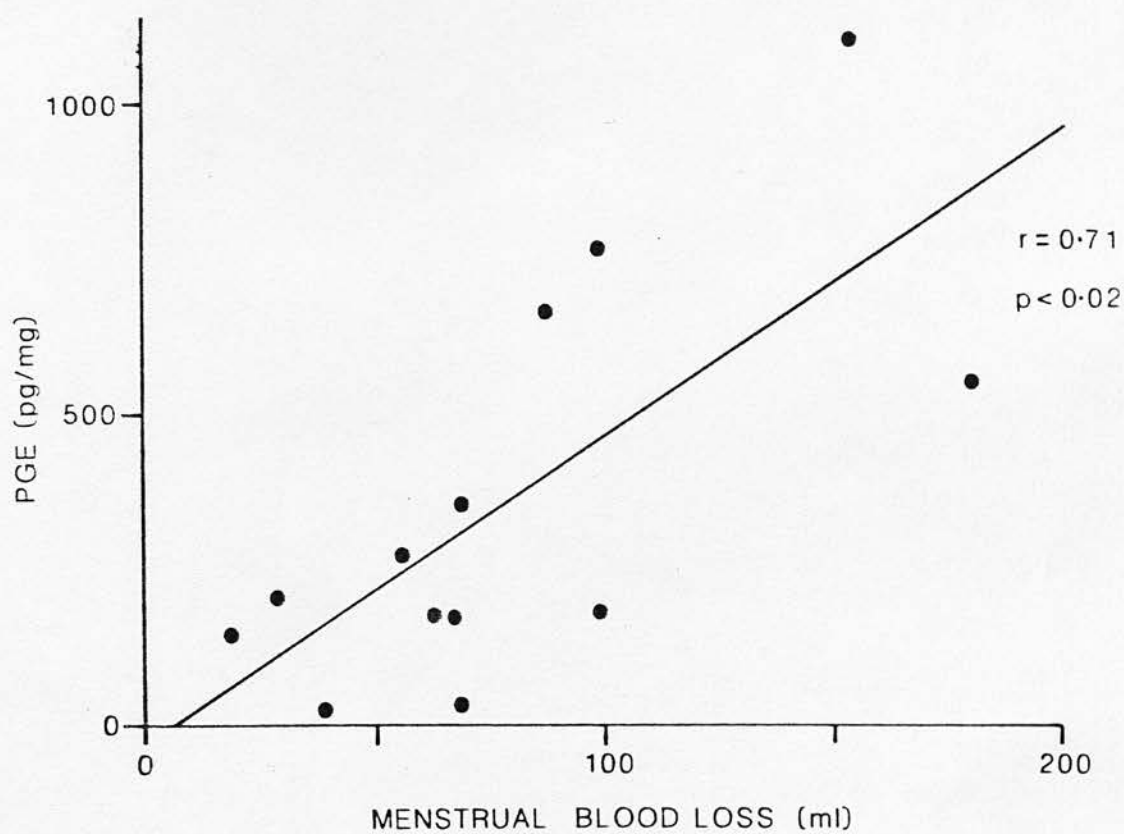
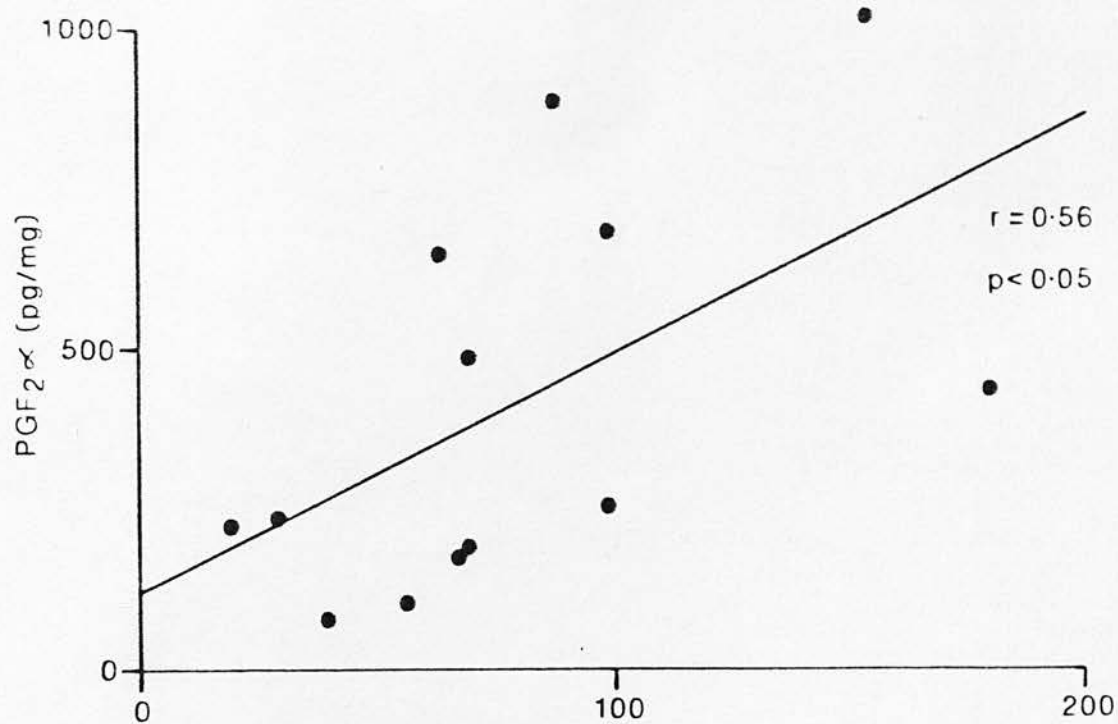
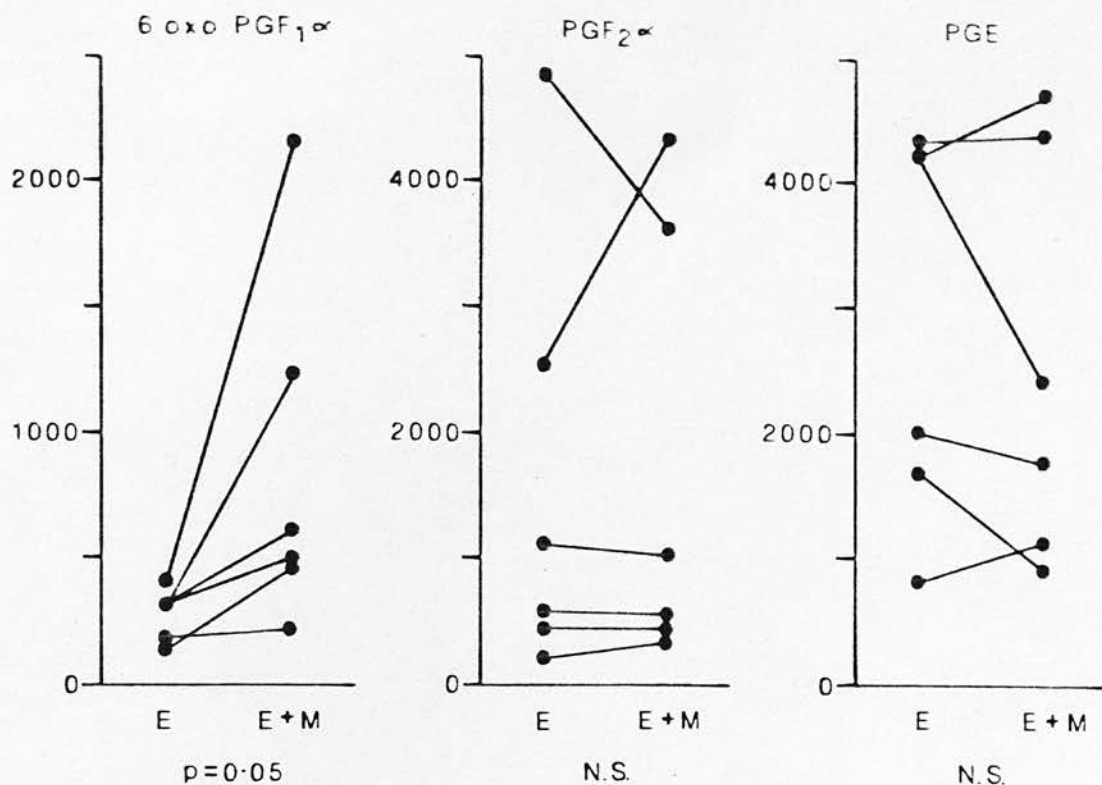


Figure 2. The relationship between menstrual blood loss and the endometrial concentration of (a) PGE and (b) PGF₂α.



PROSTAGLANDINS AS % OF TOTAL (n=6)

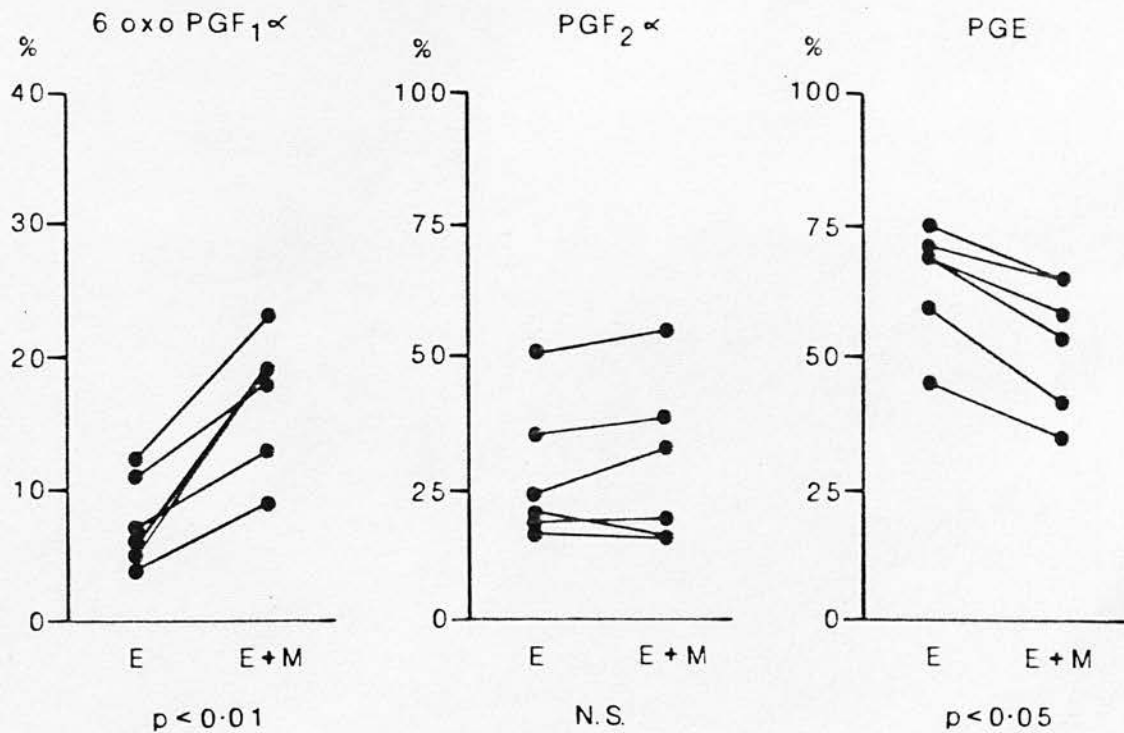


Figure 3 Combined endometrial - myometrial incubations (a) Prostaglandin production in pg/mg/hr. (b) Prostaglandin production as a percentage of "total" prostaglandin. (E - endometrium alone, E + M - endometrium plus myometrium)

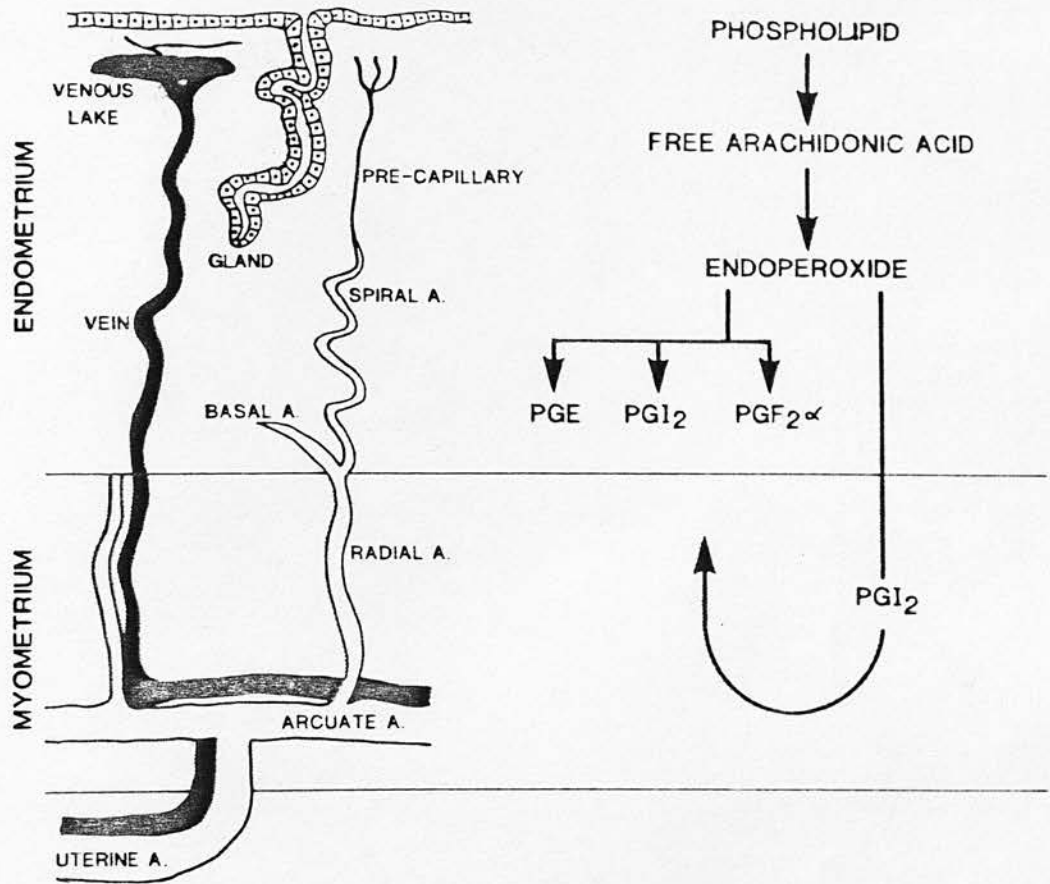


Figure 4. Endometrial prostaglandins and the uterine vasculature. (Adapted and redrawn from Baird et al, 1981, with permission).

	<u><50mls (19)</u>	<u>>50mls (26)</u>
Menstrual Blood Loss (mls)	25.2±3.0**	103.0±10.8**
Age (yrs)	39.3±1.3	40.8± 1.1
Parity (>28W gestation)	2.4±0.2	3.0± 0.3
Duration of bleeding (days)	5.1±0.3	5.1± 0.3
Cycle length (days)	28.2±1.4	26.7± 0.1
Anovular cycles (%)	3(16%)	3(12%)
Haemoglobin (g/dl)	13.6±0.1*	12.6± 0.2*
Reticulocytes (%)	1.3±0.1	1.5± 0.1

Table 1. Patient characteristics. Values are given as mean ± standard error.
(*p<0.01, **p<0.001)

	AGE(yrs)	PARITY	CYCLE	DAY
1	46	3+0	4/28	22
2	43	1+0	8/25	26
3	46	4+2	8/26	25
4	35	4+1	11/24	24
5	40	2+0	7/23	22
6	40	4+0	6/21	17

Table 2. Patient characteristics: endometrial - myometrial incubation studies.